Identification of a pKa-regulating motif stabilizing imidazole modified double stranded DNA

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Inspired by nature, the de novo design of artificial enzymes using physico-chemical principles intuition and computational methods is rapidly coming of age (1). A major design problem is the requirement to precisely engineer the position of the various functionalities required for catalysis in a productive arrangement capable of providing the basic reactivity. An alternative approach has been to simplify the design by grafting onto a simpler 'template' structure, which is selected to ensure the formation of a sufficiently rigid unit bearing the catalytic core (2,3). Using a 14mer DNA duplex as a rigid scaffold for the precise and predictable positioning of catalytic functionalities, our systems of interest can be classified as first generation hydrolase-like DNAzymes equipped with one histidine mimicking functionality based on a modified thymine nucleotide building block (T^{Im}).

Depending on the position of this peptide-like functionality, a significant increase in stability with respect to the non-modified wild type duplex due to the contribution of a single modification has been observed using UV melting experiments. In addition, an increase in pKa_H of the imidazole functionality depending on its position inside the DNA framework has been demonstrated. Most notably this is the case in the T_8^{ImH+} system, where both a significant increase in stability and pKa_H-value is perceived. Following complete ^1H NMR assignments of all modified systems, an initial view on the exact position and interactions of the imidazole moiety with the duplex is achieved using chemical shift difference and nOe-contact mapping. In a second stage, GPU-accelerated unrestrained molecular dynamics trajectories in the AMBER FF12SB force field with explicit water have been used to obtain an atomic view on the systems at hand. The overall quality and validity of the simulations was assessed by extracting relevant parameters (e.g. sampling of the α/γ conformational space (4)) for each system. Subsequently, distances between the imidazole and duplex hydrogen atoms were monitored during the trajectory and confronted with the available nOe data. Using this integrated methodology, a new pKa_H-regulating DNA motif has been identified in T_8^{ImH+} and subsequently validated in other duplexes.

When integrated into a DNA sequence, this generic motif enables a specific interaction and pKa_H-regulation of the imidazole functionality within the major groove. Simultaneous introduction with non-interacting tethered imidazoles should allow specific tuning of relative pKa_H in multiple modified systems.

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